THE KERATINOCYTE: INTER- AND EXTRACELLULAR EVENTS

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1430–1500 Y. BARRANDON
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1630–1700 L. BRUCKNER-TUDERMAN
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Desmosomal cadherins


Anti-adhesion peptides, desmocollin and desmoglein, corresponding to the cell-adhesion recognition sites of desmosomal cadherins completely but reversibly blocked alveolar morphogenesis of mouse mammary cells and disrupted positional cell sorting in aggregates of human mammary luminal and myoepithelial cells. Desmosomal adhesion appears to be as important for morphogenesis as the adherens junctions adhesion molecule, E-cadherin. Positioning of luminal and myoepithelial cells can be determined by cell–cell adhesion without a contribution from the extracellular matrix.


Expression of GFP-Dsc2a in cultured cells was used to image desmosomes. Although desmosomes are stable, fluorescence recovery after photobleaching revealed that desmocollin within them turns over rapidly. Desmosomes also persist during cell division, and internalized desmosomal halves following low calcium treatment are not re-utilized for desmosome assembly.


Dsc1 is required for strong adhesion in the upper epidermis. Its absence causes flaky skin and a striking punctate epidermal barrier defect. Desmocollin 1−/− mice show epidermal hyperproliferation, abnormal differentiation of luminal and myoepithelial cells can be determined by cell–cell adhesion without a contribution from the extracellular matrix.


Misexpression of Dsg3 in the upper epidermis by the keratin 1 promoter causes abnormal epithelial differentiation and hyperproliferation providing further evidence that desmosomal cadherins have a regulatory role in epidermis.


Ectopic expression of Dsg3 in the upper epidermis was driven by the involucrin promoter. This induced an abnormal stratum corneum with gross scaling and a lamellar histology resembling that of the oral mucosa, causing excessive lethal transepidermal water loss and early lethality. Again, desmosomal abnormal cadherin expression effects epidermal differentiation.


Demonstration that the 87-amino acid-long plakoglobin-binding domain in the cytoplasmic tail of Dsg3 is necessary to target this desmosomal cadherin to desmosomes.


Basal keratinocytes at the tips of nete ridges in human palm, a region where stem cells are believed to be located, showed low expression of desmoplakin. Isolated palm keratinocytes that adhered rapidly to collagen IV also showed reduced expression of desmosomal proteins. Cells with low surface expression of desmoglein 3 (Dsg3) exhibited high clonogenicity, colony-forming efficiency, and enhanced proliferative potential. A population containing putative epidermal stem cells could be sorted on the basis of β1-integrin-bright and Dsg3-dull fluorescence.


The newly discovered desmosomal cadherins, desmoglein 4 (Dsg4), is expressed in suprabasal epidermis and hair follicles. Mutations of the Dsg4 gene are found in families with inherited hypotrichosis and the lacoelate hair mouse. Pemphigus vulgaris sera recognize the Dsg4 protein. lab+/lab− mice having a mutation in the Dsg4 gene show hyperproliferation and abnormal differentiation in the epidermis, providing additional evidence that desmosomal cadherins regulate these processes.


The intercellular organization of desmosomal cadherins was examined by electron tomography of plastic sections from neonatal mouse skin. This analysis suggests that desmosomal cadherins form discrete groups with individual molecules interacting at their tips. Such an arrangement is consistent with a flexible adhesive interface involving standard exchange between the amino terminal subdomains, suggested by X-ray crystallography of classical cadherins.

Desmosomal plaque components


During the early stages of calcium-induced keratinocyte cell adhesion, filopodia of adjacent cells interdigitate, driven by actin polymerization and form a double row of adherens junctions, the adhesion
zipper. Adhesion is then stabilized by the formation of desmosomes between the lateral surfaces of the filopodia.


Because homozygous desmoplakin mutant embryos did not survive beyond embryonic day 6.5, the authors rescued the extra-embryonic tissues until mid-gestation by aggregation of knockout embryos with tetraploid morulae. Investigation of the fused embryos demonstrated the importance of desmosomal adhesion in the heart muscle and skin (which contain desmosomes) and the microvasculature (which does not contain desmosomes, but possesses unusual intercellular juctions composed of desmoplakin, plakoglobin, and VE-cadherin).


Conditional knockout of desmoplakin in mouse epidermis compromises epidermal cell sheet formation. The desmosomes are non-adhesive because they lack a fully formed plaque and attachment to the keratin cytokeleton. Other desmosomal components including plakoglobin, plakophilin 1, Dsc1, Dsg1, and Dsg3 still localize to cell–cell borders. Adherens junctions are also absent indicating a mutual dependence of these junctions and desmosomes.


Darier’s disease is an autosomal dominant skin disorder involving loss of desmosomal adhesion. It is caused by mutations in the ATP2A2 gene which encodes the sarc(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) isoform 2 pump, responsible for Ca²⁺ transport into the endoplasmic reticulum to maintain a low cytosolic Ca²⁺ concentration. Inhibition of SERCA with thapsigargin impairs trafficking of desmosomal components to the cell surface. In Darier’s disease, keratinocytes trafficking of desmoplakin, but not other components is significantly inhibited. Furthermore, interaction between SERCA2 and desmoplakin is demonstrated.


Activated epidermal growth receptor tyrosine phosphorylates plakoglobin. The phosphorylated plakoglobin associates with Dsg2, but not with desmoplakin, and is predominantly situated in a membrane-associated Triton-X 100-soluble pool, disassociated from desmoplakin and intermediate filaments. Thus tyrosine phosphorylation of plakoglobin may regulate desmosomal adhesion.


Plakoglobin (PG)⁺⁺⁺ keratinocytes respond to pemphigus vulgaris immunoglobulin G with keratin retraction and loss of adhesion but PG⁻⁻⁻ keratinocytes do not. Re-introduction of PG to the PG⁻⁻⁻ cells restores the response. This is the first evidence that PG plays a central role in mediating acantholysis in pemphigus vulgaris.


Plakoglobin (PG), an armadillo family protein, is a component of both desmosomes and adherens junctions. The tyrosine kinase Src phosphorylates Tyr643 of PG decreasing its interaction with E-cadherin and catenin but increasing its interaction with desmoplakin. By contrast, phosphorylation of Tyr549 increases the interaction between PG and E-cadherin and α-catenin. Thus, tyrosine kinases may have different effects on desmosomes and adherens junctions. Increased binding of PG to adherens junction components by Tyr549 phosphorylation can also upregulate β-catenin/Tcf-4 transcriptional activity.


The binding partners of the most widely expressed plakophilin, PP2, were identified. It has more binding partners than PP1, including desmoplakin, plakoglobin, Dsg1 and Dsg2, and Dsc1a and Dsc2a. Evidence is also provided that PP2 may regulate β-catenin signaling.


Both plakophilin 1 (PP1) and plakoglobin bind to desmoplakin, but PP1 binds preferentially and excludes plakoglobin binding. Desmoplakin (DP) and plakoglobin form a complex with the cytoplasmic domain of Dsg1, but full-length DP also binds to Dsg1 in the absence of plakoglobin. Both plakoglobin and PP1 are required in association with DP and Dsg1 for the formation of structures resembling desmosomal plaques.


Keratinocytes from patients with the autosomal recessive gonodermatosis skin fragility-ectodermal syndrome (OMIM 604556) lack plakophilin 1 (PP1) and possess few, poorly formed desmosomes. Re-introduction of PP1 enhanced desmosomal protein content and retarded cell migration. PP1-deficient keratinocytes in confluent cell sheets had fewer calcium-independent desmosomes than cells expressing PP1. The results suggest that PP1 has a key role in regulating desmosome assembly and adhesiveness and cell migration.


Desmosomal adhesion develops from a calcium-dependent state to a calcium-independent state in confluent epithelial cell sheets and reversion to calcium dependence occurs on wounding. The change from calcium independence to calcium dependence is accompanied by activation of protein kinase C (PKC) and localization of PKCα to the cell periphery. Antisense depletion of PKCα promotes calcium independence of desmosomes. Desmosomes in mouse tissues including epidermis exhibit predominantly, or exclusively, calcium-independent adhesion.

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Dysfunction of keratinocyte adhesion

Introduction

For normal skin function, epidermal keratinocytes need to maintain close proximity with each other. For that purpose, keratinocytes are equipped with intercellular junctions, such as desmosomes, adherens junctions, and tight junctions, that have an adhesive and signaling function. Inactivation of junctional components by mutation, autoimmune antibodies, and bacterial toxins breaches the structural integrity of embryos and adult tissues and affects proper cell morphogenesis and patterning. Recent discoveries have led to the understanding that dysfunction of keratinocyte adhesion not only may lead to bullous skin disease, but also may cause alopecia, keratoderma, nail deformities, or dry wrinkled skin.

Selected papers in order of appearance


First demonstration that defects in the armadillo protein plakoglobin cause structural defects in skin and heart and can result in embryonic lethality as a consequence of ruptured cardiac ventricles.


The paper was the first to demonstrate that an inherited mutation of a desmosomal component causes dysadhesion of keratinocytes in humans. In retrospect, the described phenotype with alopecia and teeth abnormalities in addition to fragile skin preceeded the later concept that desmosomes not only have adhesive properties and but also play a crucial role in the formation of complex tissues such as hair and nails.


Demonstration that mutations in the desmoglein 1 gene cause striate palmoplantar keratoderma. This study first supports the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex epithelia.


First demonstration that links mutations in an intracellular Ca pump with the loss of adhesion between epidermal cells and abnormal keratinization.


The paper was the first to demonstrate that the exfoliative toxins produced by Staphylococcus aureus can cleave desmoglein 1 and cause blisters in the superficial epidermis of mice that are similar to the blisters in patients with pemphigus foliaceus.


Demonstration that mutations in plakoglobin are linked to heart and skin defects in men. This study extends the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex tissues such as palmar skin and hair formation.


Demonstration that mutations in an intracellular Ca pump are linked with the loss of adhesion between epidermal cells in benign familial pemphigus.


Demonstrates an essential role for plakoglobin in mediating cytoplasmic responses to pemphigus antibodies that bind to desmoglein 3 at the cell surface.


Showed an essential role for desmoplakin in the formation of epithelial sheets and was the first to demonstrate that a lack of desmoplakin can influence adherens junction formation.


The paper compels us to consider that functional tight junctions in stratified mammalian epithelia are indeed a necessary part of the permeability barrier of the skin. Adhesion molecules, Claudins, are responsible for the barrier function. The claudin-1 null mice displayed a wrinkled skin with increased transepidermal water vapor loss leading to death within 1 day.


Demonstration that mutations in a novel desmosomal cadherin isoform are linked to hair follicle defects in mice and men. This study extends the idea that desmosomal cadherins have crucial roles in regulating morphogenesis of complex epithelia such as the formation or hair.


This study elucidated the roles of non-desmosomal acetylcholine receptors in physiologic control of keratinocyte adhesion, most probably by modulating cadherin and catenin levels and activities.

First demonstration in mice and men that the cholinergic agonists, such as carbachol and pyridostigmine bromide, may improve keratinocyte adhesion in pemphigus.


Excellent review guiding the reader into the latest concepts of cell adhesion and tissue morphogenesis regulated by desmosomes.


This study demonstrates that inhibition or blocking of the epidermal growth factor receptor promotes desmosome assembly in oral squamous cell carcinoma cells, resulting in increased cell-cell adhesion.
The epidermis, with its appendage the hair follicle, is a fascinating system to study the mechanisms controlling tissue formation and homeostasis. It is a self-renewing stratified squamous epithelium that forms the outermost layer of the skin and an essential permeability barrier, preventing moisture escape and the entry of harmful substances. Failure to form this barrier, due to immaturity of the skin in premature infants or mutation in the case of inherited disorders, can be lethal. Terminally differentiated keratinocytes, lipids, and intercellular junctions are all essential for barrier formation. The terminal differentiation program involves mitotically active basal keratinocytes detaching from the basement membrane and migrating upward toward the skin surface, from which they are shed as cornified squames. In humans, barrier function develops at 34 weeks, while in mice it occurs between E16.5 and E17.5 as the granular layer and stratum corneum form. The hair follicle is also generated during embryogenesis though a series of signals sent between the keratinocytes and the underlying mesenchymally derived dermal cells. These signals cause cell fate changes in both populations, which ultimately results in the differentiation of the hair shaft, root sheaths, and dermal papilla. The postnatal hair subsequently undergoes successive cycles of hair growth and regression. This ability of the epidermis to self-renew is dependent on the maintenance of keratinocyte stem cells. Much of our understanding of epithelial development and self-renewal has come from studying inherited human disorders and mouse mutants. Several candidate molecules have been shown to regulate epidermal development including Wnt, p63, myc, and the hedgehog family of signaling molecules. This commented bibliography lists recent papers that have contributed to our understanding of the regulatory pathways involved in the process of epidermal development.

### Bibliography


Review describing factors regulating epidermal differentiation and self-renewal.


A review of permeability barrier formation in mammalian epidermis, which also describes redundancy and compensatory mechanisms in the system.


Demonstration of that barrier formation during epidermal development is acquired in a regulated manner. The barrier was observed to occur at specific epidermal sites, then spreads around the embryo as a moving front, accompanied by multiple changes in the outer, stratum corneum-precursor cells.


The authors have previously shown that the transcription factor Klf4 is required for barrier function in the skin. In this report, they show that keratin 5 promoter-driven ectopic Klf4 expression causes competent barrier formation to occur 1 day earlier.


The targeted disruption of the desmosomal cadherin desmocollin 1 (Dsc1) causes flaky skin and a striking punctate epidermal barrier defect. This shows that Dsc1 is required for strong adhesion and barrier maintenance in epidermis and contributes to epidermal differentiation.

**Frye M, Gardner C, Li E R, Arnold I, Watt F M. Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. Development 2003: 130 (12): 2793–2808.**

Expression of c-Myc in epidermal stem cells was previously reported by Waikel et al. and Arnold and Watt in 2001 to deplete the epidermal stem cell compartment by driving keratinocytes from the stem to the transit-amplifying compartment. Myc also stimulated proliferation and differentiation along the epidermal and sebaceous lineages at the expense of the hair lineage. This investigation used microarray analysis to show that Myc activation has profound affects on the adhesive and motile behavior of keratinocytes. This reduced adhesive propensity is proposed to stimulate exit of epidermal cells from the stem cell compartment and may also affect the ability of keratinocytes to migrate along the outer root sheath, accounting for the failure of hair differentiation.


The laboratories of Bradley and McKeon in 1999 independently reported that mice lacking p63 have multiple defects that include epidermal and skeletal abnormalities. These mice do not have a recognizable epidermis but possess a thin single-cell layer covering the body, which does not form an effective barrier to prevent dehydration, and the mice die within hours of birth. This paper demonstrates that p63 isoforms regulate the initiation of epithelial stratification and suggests that p63 plays a dual role: initiating epithelial stratification during development and maintaining proliferative potential of basal keratinocytes in mature epidermis.

**Sil A K, Maeda S, Sano Y, Roop D R, Karin M. IkappaB kinase-alpha (IKKα; also called IKK1)-deficient mice surprisingly exhibit perinatal lethality and exhibit a wide range of developmental defects including impaired limb outgrowth and abnormal epithelial development. The IKKα kinase activity is required for lymphoid organogenesis and mammary gland development, whereas a kinase-independent activity is required for epithelial keratinocyte differentiation. IKKα was previously found by this group to control production of a secreted factor that induces keratinocyte differentiation. This paper shows that IKKα in the epidermis is also an important regulator of the mesenchyme. The restoration of epithelial differentiation in IKKα-deficient mice by keratinocyte-specific IKKα**

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**David M. Prowse, PhD**

Barts and The London, Queen Mary’s School of Medicine and Dentistry, Institute of Cell and Molecular Science, Centre for Cutaneous Research, Clinical Sciences Research Centre, 1st Floor 2 Newark Street, London E1 2AT, UK
expression prevented the abnormal skeletal and craniofacial morphology normally observed in these mice, an effect associated with the repression of fibroblast growth factor (FGF) family expression.


A recent examination of the in vitro and in vivo models developed to study hair follicles. The advantages and disadvantages of cell culture, organ culture of isolated follicles, and the study of natural or genetically manipulated animals are considered.


This paper demonstrates that the upper region of the outer root sheath of vibrissal follicle of adult mice contains multipotent stem cells, which respond to morphogenetic signals to generate multiple hair follicles, sebaceous glands, and epidermis. These stem cells are therefore able to generate all the lineages of the hair skin, and this requires a precise control of stem cell trafficking. Importantly, clonogenic keratinocytes in culture were also found to be closely related, if not identical, to the multipotent stem cells.


Epithelial bud development depends on epithelial mesenchymal interactions and plays an important role in the morphogenesis of several organs including the hair follicle. This research shows that remodelling of cell-adhesion junctions permits follicle formation and that this process is regulated by the Wnt and BMP extracellular signaling pathways.


An interesting paper demonstrating the effect of disrupting normal growth controls in the skin. PTEN is a tumor suppressor gene mutated in many human cancers, and a keratinocyte-specific null mutation of Pten in mice was generated using the Cre-loxP system [k5Pten(flox/flox) mice]. Skin morphogenesis was accelerated and the k5Pten(flox/flox) mice exhibited wrinkled skin because of epidermal hyperplasia and hyperkeratosis and ruffled, shaggy, and curly hair.


This paper reveals that the serine/threonine kinase Akt/PKB (which is a downstream effector of PI3K and acts as a transducer of multiple functions including cell growth, cell survival, and metabolic actions of insulin) plays a significant role in regulating epidermal proliferation and differentiation. Three isoforms of Akt (Akt1–3) have been reported in mammalian cells. Previous studies which generated Akt1 or 2 null mice did not reveal an epidermal phenotype. The double-knockout (DKO) mice reported here lack both Akt1 and Akt2 genes and exhibit severe growth deficiency, shiny skin (indicating a barrier defect), and lethality shortly after birth. The DKO mice displayed impaired skin development showing reduced interfollicular proliferation and abnormal differentiation, and reduced hair bud development.


The forkhead transcription factor FOXN1 is required for normal cutaneous and thymic epithelial development. Mutations in FOXN1 give rise to the nude phenotype in mice, rats, and man. Transient activation of FOXN1 in proliferating human primary epidermal keratocytes induced terminal differentiation. Genes promoting growth arrest, survival, and differentiation were induced by FOXN1 including the kinase Akt. Interestingly, when activated in reconstituted epidermis, FOXN1 promoted early stages of terminal differentiation, whereas Akt activation was sufficient to induce late stages including formation of the cornified layers. These results establish a role for FOXN1 in the initiation of terminal differentiation and implicate Akt in subsequent events.


Demonstration that stabilized β-catenin expressed in the basal layer of the epidermis from E15 with a keratin promoter causes de novo hair follicle formation. The new follicles show induction of Lef-1, but they are also disoriented and defective in sonic hedgehog polarization. Additionally, transgenic hair follicle proliferation can continue unchecked, causing hair follicle tumors. This paper shows that β-catenin is a key player in hair development and implicates aberrant β-catenin activation in hair tumor formation.


β-Catenin is an essential molecule in Wnt/wingless signaling, and this group have previously shown that β-catenin-deficient mice show embryonic lethality at E5.5. This paper describes a keratinocyte-specific null mutation of β-catenin in mice, generated using the Cre-loxP system, that shows that β-catenin mutation during embryogenesis blocks formation of hair follicle placodes. If β-catenin deletion occurred after hair follicles formed, hair was completely lost after the first hair cycle. During placode formation, β-catenin was found to be downstream of tgb/downd and upstream of bmp and shh. This paper demonstrates that β-catenin plays a major role in regulating stem cell fate: β-catenin signaling promotes the hair follicle lineage, while keratinocyte stem cells adopt an interfollicular fate in the absence of β-catenin.


This paper demonstrates the importance of Wnt signals for hair follicle development. In transgenic mice, an ectopically expressed diffusible inhibitor of Wnt action, Dickkopf 1, was shown to inhibit hair follicle placode formation prior to morphological or molecular signs of differentiation, and also blocked tooth and mammary gland development before the bud stage. This indicates that activation of Wnt signaling in the skin precedes, and is required for, localized expression of regulatory genes and initiation of hair follicle placode formation.


This paper demonstrates the value of utilizing a TOPGAL reporter to define activated Lef/Tcf transcription factor complexes in hair follicle morphogenesis and differentiation. Interestingly, activated Lef/Tcf complexes were observed at distinct times in hair development and cycling when changes in cell fate and differentiation commitments take place. Tcf3 was expressed in the follicle stem cells in the bulge, and TOPGAL was stimulated in this compartment at the start of the hair cycle in a manner that appeared to be dependent on stabilization of β-catenin. This shows the existence of positive and negative regulators of Lef/Tcf factors in the skin and suggests they are necessary but not sufficient for TOPGAL activation.


Overexpression of N-terminally truncated Lef1 (ΔNlefl), which blocks β-catenin signaling, results in hair loss, development of dermal

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cysts, and spontaneous sebaceous tumors. This paper further characterizes the sebaceous tumors in mice and humans and finds overexpression of the receptor PTCH and the ligand Indian hedgehog (IHH) but not Sonic hedgehog. A role for IHH in sebaceous neoplasia was supported by the observation that ΔNLefl was found to upregulate IHH and stimulate proliferation of cultured undifferentiated sebocytes.


When β-catenin signaling is disturbed from mid-gestation onward lineage commitments is profoundly altered in postnatal mouse epidermis. This paper shows that adult epidermis also has the capacity for β-catenin-induced lineage conversion without prior embryonic priming. An activatable N-terminally truncated, stabilized β-catenin estrogen receptor fusion was expressed in the epidermis of transgenic mice. β-catenin signaling was observed to trigger the active growth phase of the hair cycle (confirming a report by Van Mater et al. 2003) and de novo follicle formation in adult epidermis. Sebocyte differentiation was also affected. This shows that interfollicular epidermis and sebaceous glands retain the ability to be reprogrammed in adult life. Interestingly, long-term treatment also caused conversion of hair follicles to benign tumors resembling trichofolliculomas which regressed upon cessation of β-catenin activation.
Animal models of human skin disease

Commentary
The epidermis is a stratified epithelium which continuously regenerates from stem cells. The keratin intermediate filament cytoskeleton connects with adhesive junctions and the cornified envelope and is largely responsible for skin integrity. Many of these proteins are encoded by gene families which are differentially expressed during epidermal differentiation and regeneration. Together with human genetic studies, mouse models have been instrumental to identify disease-causing genes and have provided some unexpected and exciting insights. Loss of function studies has shown that epidermal keratins, plakins, and plakophilins as well as integrins are essential to maintain the integrity of the basal epidermis. In line, mutations in corresponding human genes underlie severe skin pathology. Genetic ablation of suprabasal keratins and other structural proteins in the suprabasal epidermis, on the other hand, have a remarkably limited influence on skin integrity. This is also true for the ablation of cornified envelope genes, at least in the mouse, either pointing to redundancy or pointing to the need to re-evaluate our hypothesis on structure–function relationships. The latter is supported by the notion that deletion of claudin-1 disrupts the epidermal water barrier without affecting the structure and composition of the lipid envelope. Also, point mutations in several genes, including keratins, produce severe phenotypes while the loss of function mutations does not. Therefore, in order to understand mechanisms leading to disease and to develop successful therapies, it will be essential to understand why epidermal protein networks are encoded by such complex gene families and how the protein networks are formed and regulated.

References
Describes the previously unnoted presence of tight junctions in epidermis and shows that the loss of claudin-1 disrupts the epidermal barrier.

Demonstrates that desmoplakin is essential for the linkage of epidermal keratins to desmosomes and for epithelial integrity.

Provides evidence that loss of alpha-catenin has profound effects on keratinocyte differentiation in vivo and in vitro.

The first demonstration that an intermediate filament mutation causes tissue fragility syndromes.

The first inducible model for epidermolysis bullosa simplex (EBS), providing evidence for lateral migration of stem cells and pointing out routes to EBS therapies.

Demonstrates the need for hemidesmosomal integrins to maintain epidermal integrity and serves as model for junctional EB (Epidermolysis Bullosa).

The first mouse model for EHK (Epidermolysis Hyperkeratosis). In conjunction with the paper of Reichelt and Magin, it demonstrates that the gain of toxic function can be far more severe than the loss of function mutations.

The most severe pathology in epidermis of all keratin knockouts, resulting from the complete absence of filaments in basal epidermis.

Provides evidence for the role of keratins as mechanotransducers. Loss of K10 leads to increased proliferation in another epidermal compartment.

Demonstrates that K17 is important for hair formation and integrity, as alopecia develops in its absence.

An unexpected observation suggesting that the major role of K6 isoforms may be to act as reinforcement keratins and not so much in wound healing.


Shows that epidermal stratification takes place in the absence of major integrins.


In conjunction with paper of Djian et al., this work shows that cornified envelope formation has many backup proteins and does not require loricrin.


See paper Koch et al.


Supports the redundancy of cornified envelope proteins and shows that in newborn mice, its formation is slightly delayed but not impaired.


A concise review.
Connexin mutations in human disease

Introduction

Gap junctions composed of connexins provide a mechanism of synchronized cellular response facilitating the metabolic and electronic functions of the cell by the direct intercellular transfer of ions and small molecules. Germline mutations in different human connexins, and indeed within the same connexin protein, can produce an array of phenotypes associated with the human ectodermal epithelium including hearing loss, neuropathy, hair growth abnormalities, and hyperkeratosis. These genetic studies also suggest that different mutations within a specific connexin have distinct effects on epidermal differentiation and the sensory epithelia of the inner ear. The association of hyperproliferative skin disease with connexin mutations supports an important function for these gap junction proteins in epidermal differentiation. The keratinocyte and its programmed cellular differentiation is proving to be an excellent model to further our understanding of connexin biology in other complex but less accessible epithelial and non-epithelial tissues including the inner ear.

References


Connexin 32 was the first disease-associated connexin and is mutated in the X-linked form of Charcot–Marie–Tooth disease. This progressive neuropathy results from myelin disruption and axonal degeneration of peripheral nerves. It is proposed that Cx32 mutations impair the diffusion of metabolites through a type of gap junction termed a ‘reflexive gap junction’ that is found between the Schwann cell body and its distal processes.


The first paper describing recessive mutations in the coding region of GJB2 encoding Cx26 associated with non-syndromic hearing loss (NSHL). Numerous subsequent studies have shown that GJB2 mutation account for a significant proportion of NSHL worldwide. Within different ethnic groups, there are specific common founder mutations that account for the majority of GJB2-related hearing loss, for example, 35delG, 235delC, and R143W in the European, Japanese, and African populations, respectively.


This paper was the first to associate connexin mutations with a disorder of epidermal keratinization. The authors describe a specific Cx26 mutation that underlies Vohwinkel’s syndrome. This disease is characterized by a honeycomb pattern of keratoderma with starfish-like keratoses on the knuckles and mild-moderate sensorineural hearing loss. Other studies have now shown other Cx26 mutations linked to keratitis-ichthyosis-deafness syndrome, Cx31 mutations associated with the skin disease erythrokeratoderma variabilis, and Cx30 mutations with the ectodermal dysplasia, Clouston’s syndrome.


EGFP (Enhanced Green Fluorescent Protein)-tagged wildtype or mutant connexin Cx31 fusion proteins were used to study mutant connexins with regard to junction assembly and function in keratinocytes. Following transfection, wildtype connexin proteins are able to traffic to the membrane and form functional gap junction plaques indicated by dye transfer. The skin disease-associated mutant proteins display limited trafficking with primarily a cytoplasmic localization. Another striking cellular phenotype was that skin disease-associated mutant Cx31 induced keratinocyte cell death, but the expression of hearing loss and/or neuropathy-associated Cx31 mutations did not.


The hearing loss phenotype seen in human Cx26 ‘knockouts’ have been replicated from gene-targeting studies in the mouse. These transgenic mice displayed cell death in the inner ear leading to degeneration of the cochlear sensory epithelium. This cell death phenotype is analogous to the keratinocyte cell death observed with skin disease-associated connexin mutations.


This study demonstrated that there are, at least, 10 connexin proteins expressed in the human epidermis with the junctional composition and cellular localization changing as keratinocytes differentiate.


The complex pattern of connexin expression in many tissue types including the epidermis suggests that there may be distinct cellular roles for individual or subgroups of connexins. This study demonstrated that intercellular channels have distinct permeabilities and selectivities for different molecules such as ATP, AMP, and IP3 depending on their connexin composition.


This paper suggests an additional role for connexins other than intercellular communication. Open connexons (hemichannels) at the non-junctional plasma membrane may play a role in paracrine signaling. These hemichannels can function within the plasma membrane of mammalian cells to regulate cell volume and that the channels are gated within the physiological range of extracellular calcium ion concentration.

These studies in HeLa cells showed that overexpressing either WT-Cx26 or WT-Cx30 could overcome the inherent trafficking defect of skin disease-associated mutant Cx26 proteins but these channels had impaired dye permeability.


In addition to epidermal phenotypes associated with the human skin disease Vohwinkel's syndrome, keratinocytes from these transgenic mice overexpressing the D66H-Cx26 mutation driven off the keratin 10 promoter displayed increased cell death. This phenotype replicates the cell death phenotype seen from previous in vitro studies.
Role of integrins in tumor invasion and metastasis

Ian R. Hart
Tumour Biology Laboratory,
Cancer Research UK Clinical Centre,
Queen Mary's School Of Medicine & Dentistry,
Barts & The London, John Vane Science Centre,
Charterhouse Square, London EC1M 6BQ, UK

Polarized cell movement and extracellular matrix degradation are important components of tumor cell invasion, and both activities are regulated by members of the integrin family of transmembrane receptors.

The integrin \( \alpha_v \beta_6 \) is a fibronectin tenascin and latency-associated peptide (LAP) of TGFB receptor, which is not detectable on normal epithelium but is neo-expressed in a range of carcinomas including oral epithelial dysplasia and oral squamous cell carcinoma (SCC), suggesting it has a role in promoting malignant behavior and tumor progression. We have used transfection and retroviral infection to create a panel of SCC cell lines expressing various levels of \( \alpha_v \beta_6 \) to examine this possibility. We found that increased expression of \( \alpha_v \beta_6 \) in malignant keratinocytes upregulates matrix metalloproteinase-9 (MMP-9) and MMP-2 expression and promotes invasion in an MMP-9-dependent manner.

Amino acids with the sequence EKQVDSLTC, which are the C-terminal residues of the integrin \( \beta_6 \) subunit, were shown to promote \( \alpha_v \beta_6 \)-dependent invasion in an MMP-9-dependent fashion. This same peptide sequence, when expressed at the cytoplasmic end of the \( \beta_3 \)-integrin subunit, was able to enhance \( \alpha_v \beta_3 \)-mediated invasive and enzymatic activity of tumor cells in an MMP-2-dependent fashion. Our results show that these 11 amino acids, when expressed at the C-terminus of the \( \beta \) subunit, are responsible for regulating the activity of invasion-promoting degradative enzymes, whereas the specific MMP involved in this cellular behavior was dependent on the context of the remainder of the \( \beta \)-integrin subunit.

It appears that integrins are moved from the invading to the retracting edge of motile cells, and this suggests that an understanding of the control of integrin internalization and endocytic recycling might permit interference with cellular motility if target molecules involved in this process can be identified.

In an effort to identify interactions between \( \beta_6 \) and putative intracellular signaling molecules, we have used the \( \beta_6 \)-integrin cytoplasmic domain as bait in a yeast two-hybrid screen of a human keratinocyte cDNA library. We found an interaction with the widely (possibly ubiquitously) expressed 34kDa protein HS1-associated protein X-1 (Hax-1). This protein appears to be involved in recycling of cell surface-located proteins and when Hax-1 was reduced in a variety of cell lines, by transfection with siRNA, transfected cells showed no alteration in morphology, growth, or adhesion to a variety of substrates but a marked reduction in cell migration toward LAP, an \( \alpha_v \beta_6 \)-specific ligand. This suggests that recycling of \( \beta_6 \) is a necessary component of \( \alpha_v \beta_6 \)-mediated migration.

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